A simple way to demonstrate sexual reproduction in the bakery mold, Neurospora

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The gardener propagates his plants either by cuttings of some sort, or by seeds. The development of a seed usually requires that the egg in the ovule be fertilized. On this account the biologist refers to the formation of a seed as due to sexual reproduction, in contrast to asexual reproduction where new plants arise directly from buds developed from leaf, stem, or root.

The fungi are very low forms of plant life, nevertheless they follow much the same laws of reproduction and inheritance that govern these processes in the higher plants. Fungi reproduce themselves asexually by means of spores of various sorts developed as natural cuttings from the vegetative growth. No act of fertilization is then involved. A square inch of the blue mold found on decaying lemons probably develops a billion or more of these spore cuttings, which serve to propagate the fungus very effectively from month to month and year to year. Sooner or later the fungus will reproduce itself sexually. This does involve an act of fertilization where two nuclei from different cells unite in a fusion. This stage is sometimes very difficult to discover and difficult to demonstrate in culture, once it is known to occur.

In an article published in Torreya, **30**: 35–39, 1930, there was given a short description of experiments with material which could be used by teachers of biology and botany to demonstrate sexual reproduction in the orange-colored bakery mold, *Neurospora sitophila*, which belongs to the class Ascomycetes. It was shown that a single spore culture of this fungus cannot produce ascocarpic fruit bodies by itself. Such strains are unisexual just as are strains of the common bread mold, *Rhizopus*, a fungus used by teachers to demonstrate sexual reproduction in the Phycomycetes. It is necessary to grow mycelia of opposite sex together in the same culture in order to obtain the sexually produced fruit bodies. Therefore, when two mycelia of opposite sex of the *Neurospora* bakery mold are grown from opposite sides in a petri dish culture, many black ascocarps are developed. Very frequently, the ascocarps are distributed over the area occupied by only one of the strains as figured in the article referred to above.

The orange-colored conidia characteristic of *Neurospora* have always been assumed to function only as asexual spores in vegetative reproduction. It has been discovered, however, that these monilioid conidia have another and quite different function in fertilization; that is, they may also function "sexually." This discovery provides the biology teacher with another and much easier method of demonstrating sexual reproduction in Ascomycetes.

It is only necessary to grow one of the unisexual strains in a petri dish culture, and then apply a spore suspension of the conidia from a mycelium of the other sex to any particular spot on the plate culture. This can be done by means of a capillary tube or fine pipette, or a fine water color brush. When one puts pollen on the receptive stigma of a flower we call this act pollinating. Therefore, when one places the conidia of the bakery mold on the receptive organs developed on the mycelium we may refer to the act itself as conidiating.[1] Within twenty-four hours after conidiating the receptive bodies one can see exactly where fertilization has taken place, resulting in the rapid formation of the ascocarpic fruit bodies. With a hand lens or low power dissecting microscope, the student can readily distinguish individual ascocarps, such as were shown in figure 2, page 38, of the article in Torreya referred to above. Perithecia (ascocarps) are produced only where the conidia come in contact with the receptive bodies of which there will be thousands scattered all over the culture ready to be fertilized. Figure 1 shows a photograph of a plate culture where the fertilizing conidia were originally applied as though one were printing the letters with invisible ink. The receptive bodies that were not fertilized are not visible in this picture except perhaps showing as a fine powder at the center of the culture. The young ascocarps, however, were readily visible 24 hours after the conidia were applied.

Curiously enough the conidia from the culture producing the receptive bodies will not bring about fertilization when applied

[1] The writers are indebted to Dr. H. A. Gleason for suggesting this very descriptive term "conidiate." You pollinate why not conidiate?

to the bodies formed in the *same* culture. It is only when they are taken from a mycelium of the opposite sex that they will be stimulated to develop the ascogenous elements. This brings up the question as to what we mean by sex in the fungi. Here are two mycelia, or two plants we may say, both apparently alike morphologically, producing the same kind of structures, yet they act only reciprocally in sexual reproduction. Of the many races of this fungus that one could obtain from ascospores

Figure 1. Controlled fertilization in the bakery mold. Sexual reproduction only where the fertilizing elements (the orange-colored spores) were placed in contact with the receptive bodies (Fig. 2). The black bodies making up the letters represent something like 4000 sexually produced ascocarpic fruit bodies.

in culture, there would normally be only two kinds when it comes to sexual reproduction. It is convenient to refer to the individuals of the two groups as being of opposite sex. As a matter of fact, it is impossible to apply accurately the terms male, female, sex, and sexuality in connection with these lower plants.

The little sclerotioid receptive bodies (Fig. 2) are readily visible under the lower power of the microscope, so that their location can be marked definitely. They can then be watched, after conidiation, to see that they are the bodies which actually become fertile perithecia. Figure 2 shows seven such receptive bodies as they appeared at the time they were conidiated; and Figure 3, the same bodies twenty-two hours later. The second picture shows that only three of the seven bodies became fertilized. The other four grew no further because they were not fertilized.

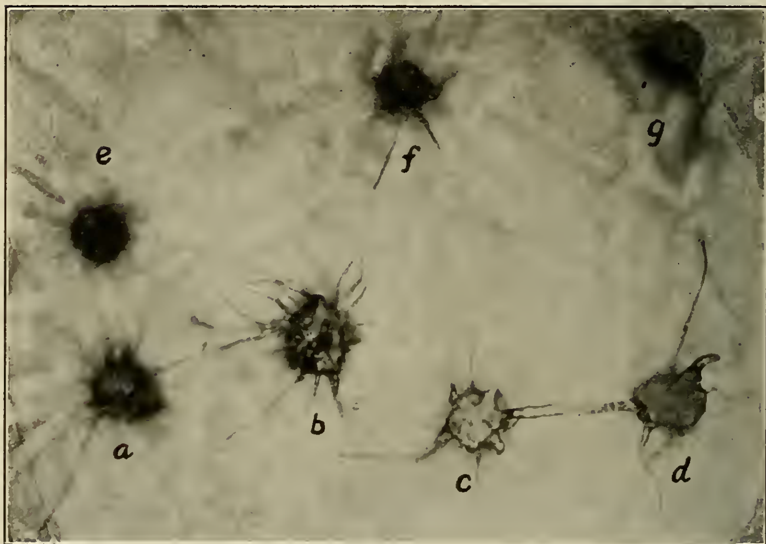

Figure 2. Seven receptive bodies of the bakery mold, ready for fertilization, at 5:30, P.M. At this time the fertilizing elements (the orange-colored spores) were placed on the bodies in a drop of water.

The story of the origin of the ascocarps usually given us in text books of botany implies that there is some sort of oogonium, ascogonium or female receptive body which must be fertilized *before* the ascocarp will begin to grow. This work on *Neurospora* shows that fertilization can be brought about *after* the ascogonial coils are well covered with protecting sclerotioid elements. That is, incipient ascocarps develop to some extent before fertilization. The appearance of the hair-like growths from these bodies (Fig. 2) would suggest that they are all trichogynous receptive elements. As a matter of fact, only one of these outgrowths is the trichogyne end of the ascogonium which is concealed within the incipient perithecium. That a transfer of nuclei from the conidia does take place can be readily demonstrated. Whenever an albino race of one sex is fertilized by applying the orange-colored conidia of the opposite sex from a normal race, half of the progeny ascospores that result are albinistic, and the other half contain factors for the production of orange-colored conidia. The segregation of the factors for sex and the factors for conidia takes place according to Mendel's laws.

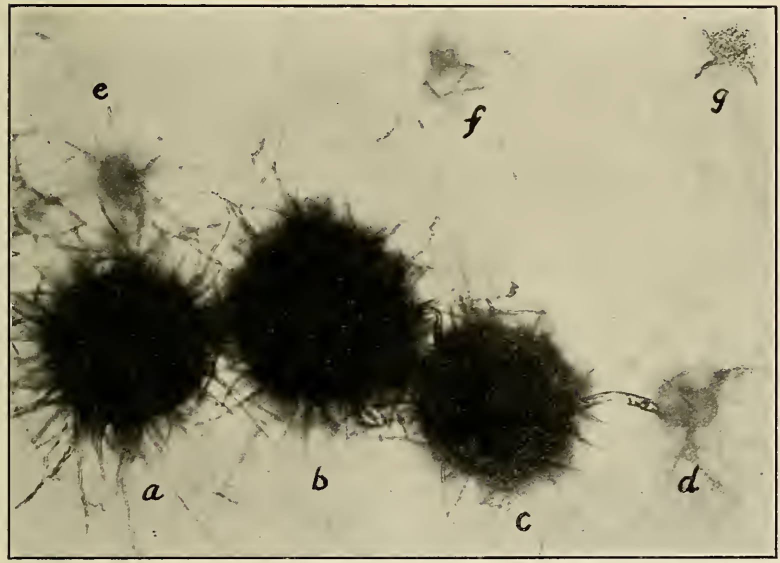

Figure 3. The same seven receptive bodies shown in figure 2, twenty-two hours later, photographed at a higher focus but with the same magnification. Bodies a, b, and c, as seen by their larger size, were successfully fertilized. The others, not being fertilized, made no further growth, and being smaller, they are necessarily out of focus in the picture.

Any mycelium of the bakery mold under favorable conditions can be made to produce minute spermatium-like microconidia (microspores). It has been assumed that these microconidia are male organs, constitutionally different from the orange-colored monilioid conidia borne on the same mycelium.

It has been proved, however, that the microconidia, if placed in the right environment, slowly germinate, and eventually produce perfectly normal mycelia. If the microspore came from a mycelium that also produced monilioid conidia, then its mycelium will produce monilioid conidia. Functionally it isn't male any more than any other structure produced by that mycelium is male. Microspores, monilioid conidia, ascogonia and "sclerotia" are merely specially differentiated structures; as such they are better able to function in certain ways and thus bring about certain developments. That is, as just stated, one is genetically or inherently no more male or female than the other bodies developed on the same mycelium, because in each lies the potentialities of the whole race. Blakeslee's final conclusions are that in the Mucoraceae morphologically the + and − strains do not represent male and female strains. Nevertheless, they are of opposite sex in their reactions. The difference in the size of the fusing gametangia in *Zygorhynchus* or in *Dicranophora* is no criterion of maleness and femaleness. The larger gametangium is + in one case and − in the other. There are certain sex factors in heterothallic species, however, which determine whether or not two mycelia will unite in sexual reproduction. This apparently is essentially the situation in *Neurospora sitophila* where a mycelium is neither male nor female. It produces coiled structures, monilioid conidia, microspores, sclerotia, etc. Genetically they are all exactly alike barring mutations which may have occurred during the life of the culture. The ordinary monilioid conidia can function either in fertilization directly, or as asexual spores in their vegetative function. The microspores can function not only as vegetative spores, but also as "spermatia" in sexual reproduction. There are few cases where the student can be shown so clearly that an organ can be one thing morphologically, and function in an entirely different capacity. It might not be out of place to point out to him that when we use the terms, male, female, sex, sexuality, in discussions involving the lower plants such as the fungi, we are not always consistent. We certainly cannot use these terms in the sense in which they are applied to the animals or to the higher plants. For example, the microconidia of *Neurospora* are homologous with the so-called spermatia of the lichens and other fungi, and probably with the spermatia of the red algae, but they are in no sense sperms. In the red algae and the fungi it is the nucleus (together with whatever cytoplasm that passes on with it) that is, if anything, comparable or analogous to a sperm. When, therefore, one places either the orange-colored conidia or the microconidia of *Neurospora* on the receptive organs, as was done in the experiments described above, he really does not spermatize, he merely conidiates or microconidiates (spermatiates), just as one pollinates.

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